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STUDIES IN THE PHYSIOLOGY OF COMMENSALISM. 2. THE POLYNOID GENERA ARCTONOË AND HALOSYDNA

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In earlier experiments (Davenport, 1950), it was demonstrated that the specificity of the commensal polynoid worms *Arctonoë fragilis* (Baird) and *A. pulchra* (Johnson), respectively inhabiting the starfish *Evasterias troschelii* Stimpson and the sea-cucumber *Stichopus californicus* (Stimpson), could at least in part be explained by a positive response which these commensals showed to their hosts. On the basis of these tests it was concluded that "certain echinoderm hosts produce a specific diffusible substance that acts as a powerful attractant for their commensals."

Welsh (1930, 1931) had demonstrated that similar agents govern the specificity of certain clam-acarine partnerships. In the course of his work these chemical agents were found to be relatively stable. Boiling for five to ten minutes as well as putrefaction for a week at 37.5° C. had little effect on the material acting on the mites. Beyond this, no further investigation of the nature of the substance was carried out.

To the writers it also seemed of primary importance to determine whether or not the attractants involved in echinoderm-annelid partnerships are stable, as a first step in an investigation of the nature of these substances. In addition, questions had arisen as to the source of the attractants in the host. The necessity for investigation of the factors determining the specificity of further commensal partnerships involving diverse phyla was, as always, present.

In the summer of 1950, experiments designed to attack some of the above problems were conducted at the Oceanographic Laboratories of the University of Washington at Friday Harbor, under grants from the American Philosophical Society and the Society of Sigma Xi. The writers wish to express their appreciation to the Societies and to the Director and staff of the Laboratories for their generosity and assistance.

The Genus Arctonoë

Material

Evasterias and Stichopus with their attendant commensals were collected in the same localities and in the identical manner as described in earlier work (Davenport, 1950). For one series of experiments, however, a large number of Evasterias averaging the size of a silver dollar (3–15 cm.) were collected on a point near the village of Olga on Orcas Island. These immature starfish did not seem to have as many commensals (5 out of 41) as a comparable population of adults, on a third or more of which one would find worms. At this particular locality one could collect great numbers of immature stars, but extremely few adult ones; it would seem that immature populations are as locally distributed in the Sound inter-tidal as are adult ones.

Methods

The equipment described for earlier experiments (Davenport, 1950) was used, and an additional apparatus was also developed so that two experiments could be conducted simultaneously. The aquaria and the Y tubes of this new apparatus were constructed of lucite, the aquaria being built of one-foot square, ¼″ lucite and the Y tubes of ¾″ tubing. In order to reduce the possible effects of light, the Y tubes were painted with an opaque black paint along the sides of the stem and arms, leaving a median unpainted area for observation of the worms.

Methods of handling the animals, conducting experiments and recording data were as described previously, except that it soon became clear that in order to demonstrate a positive response, it had neither been necessary to separate the populations of commensals from their hosts for a period of time, nor to keep hosts for a time in non-circulating sea-water before testing so that attractant could accumulate. In control tests it was shown that worms which had just been removed from their hosts would give a series of strong positive runs if trials were commenced from 10 to 15 minutes after a host was placed in the test aquarium in fresh sea-water.

Observations

Are attractants stable? Suppose a star is placed in a test aquarium for a time and then removed; how long does the water retain its attraction for commensals?

Experiment No. 1. A host Evasterias was placed in non-circulating aerated sea-water overnight. After 18 hours the starfish was removed. The water in this aquarium was kept at 12.5° C. and continuously aerated for another 24 hours, at the end of which time it was tested:

No. of trials	16
No. of runs	10
No. of runs into Evasterias arm	4
No. of runs into sea-water arm	6
No. of failures	6
% failures	37%
No. of negative trials (6 plus 6)	12
% negative trials	75%
No. of *A. fragilis* used	8

At the end of these runs the starfish was returned to the test aquarium and three hours later, control tests were run:

No. of trials	11
No. of runs	10
No. of runs into Evasterias arm	9
No. of runs into sea-water arm	1
No. of failures	1
% failures	9%
No. of negative trials (1 plus 1)	2
% negative trials	18%

A comparison of the ratios of the number of runs into the Evasterias arm to the number of negative trials, both before and after the host was returned to the test aquarium (4/12 vs. 9/2), gives a χ^2 of 8.4 and a $P < .01$. The data indicate that the behavior of the commensals differed after re-introduction of the host; this agrees with the observed difference in behavior (increase in activity), there being a return of the normal observable response to the presence of the host in the system.

It would appear that water from which a host had been absent for twenty-four hours no longer retained its attraction for commensals.

In similar tests, water which had housed a star was shown to have lost its effectiveness five hours after removal of the star.

Experiment No. 2. Procedure was next reversed so that controls were run first and the star then removed; *i.e.*, a star was placed in the test aquarium, a number of trials made, the star removed and further trials immediately made. In this experiment the starfish was removed simply by lifting it from the test aquarium. However, considerable difficulty was encountered in removing the starfish, which clung firmly to the walls of the aquarium. Removal resulted in the tearing off of a number of tube feet which remained attached to the aquarium walls. The effect of such removal was immediate and dramatic.

Control:

No. of trials	18
No. of runs	18
No. of runs into Evasterias arm	16
No. of runs into sea-water arm	2
No. of failures	0
% failures	0%
No. of *A. fragilis* used	6

Host removed:

No. of trials	6
No. of runs	0
No. of failures	6
% failures	100%

This effect was so immediate, the behavior of the worms being so completely altered (lack of activity, reversal of position), that the host was returned to the apparatus and the testing continued. Re-introduction of the star at this point *did not* alter the negative behavior of the worms, even after the passage of 2–3 hours:

No. of trials	12
No. of runs	3
No. of runs into Evasterias arm	0
No. of runs into sea-water arm	3
No. of failures	9
% failures	75%

In this experiment, great care was taken so that no other factor was involved than the difficult removal of the host. The authors feel convinced that the immediate change in the behavior of the commensals was effected by removal of the host alone. Could the same experiment be conducted so that removal of the host could be accomplished without as much disturbance to it?

Experiment No. 3. This experiment resembled the preceding except that when the host star was removed, it was given a quick and sudden shock so that it came freely and easily from its position in the aquarium.

Control:

No. of trials	19
No. of runs	19
No. of runs into Evasterias arm	18
No. of runs into sea-water arm	1
No. of failures	0
No. of negative trials (1 plus 0)	1
% negative trials	5%

Host removed:

No. of trials	19
No. of runs	16
No. of runs into Evasterias arm	13
No. of runs into sea-water arm	3
No. of failures	3
No. of negative trials (3 plus 3)	6
% negative trials	31%

In this experiment the effect of removal of the host on the behavior of the worms was not as marked as in the preceding one; it would appear that if the host is easily and delicately removed, some attraction for commensals remains in the water. A comparison of the two proportions of choice above (18/1 vs. 13/3) shows no statistically significant difference, but the appearance of a number of failures shortly after the host was removed and the observed decrease in activity and movement of the worms when on trial indicated that some change had occurred. It could not be determined whether this was the result of extreme instability of the attractant or of the slow dilution of whatever attractant remained in the test aquarium, occurring when it was necessary at the beginning of every trial to equalize the pressure in the arms of the Y by balancing the water in the two aquaria (*cf.* Davenport, 1950).

In view of the difficulty of removing the host from the test aquarium without negating the response, and because of the relatively short time that water which had housed the host retained its attraction, it appeared at this point difficult to design experiments to determine something about the nature of the attractant by testing its heat-stability, solubility in ether or alcohol, adsorption on charcoal, etc. One experiment, however, was conducted which gave some evidence as to the physical nature of the attractant.

Experiment No. 4. A single host Evasterias was placed in sea-water inside a ten-inch Visking Corp. dialyzing bag. This was left open at the top and in turn suspended in sea-water in the test aquarium. Bubblers were placed both inside and outside the dialyzing bag, giving thorough aeration and mixing. After the passage of two hours, trials were commenced:

No. of trials	12
No. of runs	3
No. of runs into Evasterias arm	1
No. of runs into sea-water arm	2
No. of failures	9
No. of negative trials (9 plus 2)	11
% negative trials	91%
No. of *A. fragilis* used	6

At the end of these tests which clearly indicated no attractant to be present outside the bag, the bag was carefully split open with a razor and the contained sea-water and starfish allowed to escape into the test aquarium:

No. of trials	12
No. of runs	12
No. of runs into Evasterias arm	12
No. of runs into sea-water arm	0

This experiment would indicate that the attractant does not readily diffuse through the dialyzing bag and that therefore it is probably not a relatively simple molecule.

Experiment No. 5. In an effort to determine whether the attractant may diffuse through such a membrane only very slowly, on the day following the preceding experiment the same starfish was placed in a bag and then suspended in the test aquarium for eleven hours before runs were made:

No. of trials	12
No. of runs	4
No. of runs into Evasterias arm	2
No. of runs into sea-water arm	2
No. of failures	8
No. of negative trials (8 plus 2)	10
% negative trials	83%
No. of *A. fragilis* used	6

The contents of the bag and the starfish were then released into the test aquarium:

No. of trials	13
No. of runs	7
No. of runs into Evasterias arm	4
No. of runs into sea-water arm	3
No. of failures	6
No. of negative trials (6 plus 3)	9
% negative trials	69%

This experiment gave no indication whatever as to how much of the attractant had diffused after the passage of eleven hours, since the performance of the commensals after the bag had been opened into the test aquarium was as negative as it had been before the bag was opened. However, the failure to respond after the opening of the bag was in itself interesting. In view of the fact that many control starfish had given positive series of runs after isolation in non-circulating, aerated sea-water for as long as 15 hours, the experiment gave evidence that suspension of a star in a bag for as long as 11 hours, in spite of constant aeration and no appreciable rise in temperature, constituted some disturbance which either prevented release of the attractant by the star or caused the star to produce substances which masked the effect of the attractant.

Can the source of the attractants in the host be localized?

Experiment No. 6. Is the integument of Stichopus the source of the attractant for *A. pulchra?* A single Stichopus was completely eviscerated and the oral and aboral regions removed, leaving a large rectangular strip of integument including the longitudinal musculature. This was thoroughly washed in fresh running sea-water in order to remove traces of the visceral contents and then placed overnight in aerated but non-circulating sea-water at approximately 12.5° C. Both immediately prior to testing and at the end of the experiment some hours later, the integumental strip was still fresh and responded to stimulation or stretching by contraction.

No. of trials	41
No. of runs	23
No. of runs into Stichopus arm	12
No. of runs into sea-water arm	11
No. of failures	18
% failures	44%
No. of *A. pulchra* used	12

Experiment No. 7. In this experiment, a check on the preceding, the integuments of three large Stichopus selected at random were washed for some seven hours in fresh running sea-water to insure removal of any contaminants from the viscera, and in the hope that injury substances might be eliminated. The integumentary strips were placed in non-circulating, aerated sea-water at 12.5° C. overnight before testing. Both prior to and at the end of the experiment the three strips were fresh and responded to stimulation by contracting.

No. of trials	15
No. of runs	6
No. of runs into Stichopus arm	5
No. of runs into sea-water arm	1
No. of failures	9
% failures	60%
No. of *A. pulchra* used	6

Combining the data from experiments 1 and 2:

No. of trials	56
No. of runs	29
No. of runs into Stichopus arm	17
No. of runs into sea-water arm	12
No. of failures	27
% failures	48%

It can be seen in the above data that in these two experiments the distribution into the two arms did not significantly differ from a random one, while almost half the trials resulted in failures. It would appear from these experiments that *A. pulchra* will not respond to washed, eviscerated host integument.

Experiment No. 8. A. pulchra is sometimes found within the oral aperture of Stichopus; does the digestive system of the host produce an attractant? The entire viscera of a single large Stichopus was removed, remaining attached to an integumentary ring including the tentacles. This preparation was thoroughly washed, isolated in aerated, non-circulating sea-water overnight and tested.

No. of trials	25
No. of runs	13
No. of runs into Stichopus arm	2
No. of runs into sea-water arm	11
No. of failures	12
% failures	48%
No. of negative trials (11 plus 12)	23
% negative trials	92%
No. of *A. pulchra* used	12

From the proportion of runs into the Stichopus arm to the number of negative trials (2/23), it would appear that the commensals were repelled by the preparation, indicating, perhaps, the presence of digestive juices or injury products.

Experiment No. 9. Does the integument of Evasterias produce an attractant for *A. fragilis?* A freshly collected Evasterias was opened along the aboral surface of the arms and the entire viscera removed. This preparation was washed for two minutes in fresh running sea-water and tested.

No. of trials	26
No. of runs	15
No. of runs into Evasterias arm	9
No. of runs into sea-water arm	6
No. of failures	11
% failures	42%
No. of *A. fragilis* used	6

It appears from the above data that the cleaned, eviscerated integument of a host Evasterias has no attraction for commensal *A. fragilis.*

Experiment No. 10. A. fragilis are often found lying close to the mouth of the host star; could the digestive system give rise to the attractant substance? The entire digestive systems of three host stars selected at random were removed, ground up together in a small quantity of sea-water in clean sand and the preparation placed in the test aquarium.

No. of trials	18
No. of runs	6
No. of runs into Evasterias arm	1
No. of runs into sea-water arm	5
No. of failures	12
% failures	66%
No. of negative trials (5 plus 12)	17
% negative trials	94%

The very high percentage of negative trials indicates that the preparation repelled the commensals.

The above experiments (nos. 6–10) gave evidence that it will be difficult to localize the source of the attractants by dissection techniques. However, in the latter part of the summer, after experiments to determine the source of attractants had been conducted, it became apparent that for some unknown reason host-species individuals (both Evasterias and Stichopus) were beginning to show considerable variation insofar as attraction for commensals was concerned. Occasionally, specimens taken from the live-boxes as well as some freshly collected individuals exhibited no attraction whatever, in spite of appearing perfectly healthy and normal. Ideally, therefore, Experiments 6–10 should have been conducted with material dissected from whole animals with which immediately preceding control runs had been successfully made. Lack of time prevented repetition of the experiments using this control. However, as has been stated, Experiments 7 and 10 were controlled to the extent of using three preparations selected at random, while in Experiments 8 and 10, production of attractants by viscera, if still occurring, could not be demonstrated, since the preparations clearly repelled commensals.

Experiment No. 11. The circumstance of discovering a locality where large numbers of immature Evasterias could be found made possible an experiment to give evidence as to whether the young stars exert as strong an attraction for commensals as do older ones. Forty-one small Evasterias weighing a total of approximately 517 grams were placed in non-circulating, aerated sea-water overnight. The commensals used were taken from large stars.

No. of trials	13
No. of runs	13
No. of runs into Evasterias arm	6
No. of runs into sea-water arm	7
No. of failures	0
No. of *A. fragilis* used	6

Tests of commensals against sea-water only (Davenport, 1950) showed that out of 21 trials, 8 were failures and the commensals were very inactive. In the above experiment the absence of failures and the observed behavior of the worms indicated the presence of attractant, but the random distribution into the arms would seem to indicate that concentrations may not have been high enough to have a strong positive effect on the choice of the commensals.

The Genus Halosydna

Material

The commensal partnership exhibited by the terebellid *Amphitrite robusta* (Johnson) and the polynoid *Halosydna brevisetosa* Kinberg would appear to be a most valuable one for study. In the first place the animals can very readily be collected at medium low tides at numerous localities near the laboratories. The material used in the following experiments was found under boulders at Jones Beach, San Juan Island; Minnesota Reef, San Juan Island; a point on Wasp Passage, Orcas Island; and a point near the village of Olga, Orcas Island. Almost any boulder-strewn intertidal area in which there is a soft muddy-sand substrate is likely to harbor numbers of these interesting terebellids and their commensals. Often under the same boulders and in *immediate contact* with the tubes of Amphitrite which house both host and commensal can be found the tubes of the green terebellid *Eupolymnia crescentis* Johnson with its commensal crabs (*Pinnixa* sp.). No Halosydna were found commensal in these neighboring Eupolymnia tubes, nor have they ever been so described. Miss Pettibone (1947) has also found the scale worm in association with *Thelepus crispus* Johnson in Puget Sound. At the same time, free-living and morphologically different *Halosydna brevisetosa* can be readily collected among the byssus threads of mussels and among the roots of the basket grass, Phyllospadix (rocky coast of west side of San Juan Island; Iceberg Point, San Juan Island, etc.).

Here, therefore, are forms in which it should be possible to design experiments comparing the behavior of free-living and commensal members of the same species in relation to the two host forms, and in relation to relatives of the host forms often associated with them but never housing the commensals.

Initially it was imperative to determine whether the same type of chemical attraction existed between Amphitrite and Halosydna as had been demonstrated in echinoderm-annelid partnerships.

Methods

Commensal teams of these animals were either kept all together in a large open white tray of circulating sea-water, or host and partner were kept together in finger-bowls immersed in sea-water in a large tray. It was found that as long as there was constant and rapid water change and aeration, the animals would stay in good condition for many days; experiments were conducted, however, with recently collected material. The method of conducting experiments was identical to that used for the echinoderm-annelid partnership.

Observations

In two separate experiments, using different animals, it was found impossible to demonstrate any attraction for commensal Halosydna by sea-water containing host *Amphitrite robusta*.

Experiment No. 1. Nineteen Amphitrite were placed in non-circulating aerated sea-water overnight.

No. of trials	58
No. of runs	50
No. of runs into Amphitrite arm	26
No. of runs into sea-water arm	24
No. of failures	8
No. of Halosydna used	8

Experiment No. 2. Eighteen Amphitrite were placed in non-circulating aerated sea-water overnight.

No. of trials	60
No. of runs	59
No. of runs into Amphitrite arm	35
No. of runs into sea-water arm	24
No. of failures	1
No. of Halosydna used	6

In this experiment, after 42 trials had been run giving a random distribution, a large number of pieces of the sandy tubes of the hosts were placed in the test aquarium with them. The presence of this tube material in no way altered the subsequent behavior of the commensals, their distribution in the arms of the Y still being random.

Combining the data for Experiments 1 and 2:

No. of trials	118
No. of runs	109
No. of runs into Amphitrite arm	61
No. of runs into sea-water arm	48
No. of failures	9

In these experiments, it was clear that water coming from a number of host terebellids had no attraction for the commensal Halosydna. It therefore appeared necessary to attack the problem of the agency governing this partnership from some other direction; perhaps chemical factors are not involved. It was impossible to do more than to initiate this work with some brief and qualitative experiments.

It was first determined that although slightly negative to light, commensal Halosydna are not strongly negatively phototactic. A glass aquarium was painted an opaque black, leaving a small transparent window at one end. A lamp placed at this window enabled one to flood the bottom of the tank with light from one end. Worms were observed through a dark but transparent glass cover. If a number of Halosydna were placed in the aquarium with the light on, they were observed to make more or less random movements and after the passage of some minutes, the majority congregated at the dark end of the aquarium.

Observations were also made on the behavior of the commensals when placed in open white trays with a host animal. Such commensals moved slowly about the tray in a random manner until they came in contact with one of the long extended tentacles of the host. This contact caused commensals to move directly toward the Amphitrite along the tentacle until they came in contact with the body of the host, whereupon they wrapped themselves around the body of the host. This response after contact with the tentacle was most marked.

It remains to investigate whether similar responses of free-living Halosydna to Amphitrite and of commensal Halosydna to non-host terebellids exist.

Conclusions

Considering the experiments with the echinoderm-annelid partnerships as a whole, it would appear that a rather tenuous bond exists between host and commensal.

Initially, it is clear that the attractant from Evasterias is relatively unstable. Experiments gave evidence that this substance did not readily diffuse through dialyzing membranes.

A number of different experiments seemed to indicate that any disturbance to the host is likely to negate the response of commensals. Violent removal of the host from the test aquarium apparently brings about the release of substances which not only negate the response but actually repel the commensals. Suspension of a host in a dialyzing bag for eleven hours negated the response of worms to this host after the host had been released. It seemed impossible to demonstate any response of commensals to parts of their hosts. These results are quite in agreement with those obtained (Davenport, 1950) in tests on the Luidia-Arctonoë partnership in which it was impossible to confine a host Luidia in a test aquarium without autotomy of arms occurring, and in which failure of commensals to respond to the host was attributed to the consequent release of repelling injury substances, as indicated by the high number of negative trials (27/35).

Finally, the tenuity of the bond is also indicated by the variation in the power of attraction among individual Evasterias and Stichopus, which first became evident about the middle of the summer during which these investigations were made. Undoubtedly the inability of hosts to attract commensals resulted in some cases from confinement under slightly abnormal conditions in live-boxes, for some individuals that had earlier in the summer given strong series of positive runs later lost their attraction. However, the failure of a few freshly collected hosts to attract commensals is unexplained. This may have been the result of unknowing adverse treatment in handling, but perhaps there is fluctuation in the ability of hosts to attract.

Two possibilities concerning the agency governing the echinoderm-annelid relationships would seem to exist. One is that the specificity of the host-commensal relationship may depend upon the conditioning of commensals to a single, relatively unstable attractant, which is rapidly altered, or the effect of which is easily masked by other substances produced by disturbance or damage to the host animal. It may be possible, though it does not seem reasonable to assume it, that the production of the substance on which specificity depends would suddenly cease if hosts were dissected or disturbed or simply kept under conditions differing so little from natural ones as those in the live-boxes. It would seem more likely that attractants are readily masked by other substances, although this was certainly not the case in Welsh's (1930) experiments in which clam tissue retained its effect on the phototaxis of commensal mites even after decomposition at 37.5° C. for a week. In this particular partnership Welsh's experiments indicated the presence of stable and powerfully-acting agents which could not be altered or masked by decomposition; from this he reasoned that the effective substances might be decomposition products of the mucus or other proteins from the gills.

A second possibility for the echinoderm-annelid partnership should be kept in mind, although for it there is admittedly little experimental evidence at this time. It is possible that commensals may be conditioned to a pattern of chemical stimuli from the host and that since this pattern is made up of the sum of a number of factors it is therefore a delicate one which may be altered by a change in any one of the factors with the result that the response of commensals is negated.

At any rate the relative instability of attractants as well as the ease with which their effect may be negated by any disturbance to the host will obviously make more difficult an analysis of their physical and chemical characteristics.

In the echinoderm-annelid relationships the adaptive value of this conditioning to a substance or pattern of substances which is relatively unstable and which may be easily masked is clear. Both Evasterias and Stichopus are generally found in association with numbers of their fellows. Any circumstance bringing about dangerously abnormal conditions for the host (attack and autotomy, evisceration, etc.) may break the bond for the commensal and conceivably allow it to search out another normal host. To test this hypothesis experimentally should be possible.

The experiments with Halosydna and Amphitrite need not be taken to indicate that chemical factors are unimportant as agents of this commensal relationship. It is clear that no response could be demonstrated with the Y-tube apparatus. The response of the commensals to the tentacles of the terebellid may indicate the presence of a relatively insoluble attractant closely bound to the tentacles of the host, or the response may be purely tactile, chemical factors not being involved at the stage of the partnership experimented with. At any rate, these experiments, as well as those with the echinoderm-annelid partnerships, emphasize the importance of thorough investigations of the life-histories of commensals in relation to life-histories of hosts, in an effort to determine time and manner of adaptation to hosts, etc. In this regard Dr. Pettibone (1947) has found young Halosydna not more than 7–9 mm. long in association with young Thelepus; she believes that commensalism in Halosydna may start early and that host and commensal grow up together. This is in agreement with the observations of the authors, who found only large Halosydna commensal with the large Amphitrite of the collecting station near Olga. However, in the Evasterias-Arctonoë relationship, Miss Pettibone and the writers have often found small (4–5 mm. in length) commensals, sometimes in numbers, on large hosts and often on one host commensals of differing growth stages. Greater numbers of large stars possess commensals than do immature stars; occasionally, however, one will find commensals from 3–4 cm. long on hosts that are not more than 4 cm. in diameter. In such a partnership the commensal will take up space in the ambulacral groove almost all the way across the starfish. It would therefore appear that in the echinoderm-annelid relationship a number of generations of commensals may pass their life on the same host; we also find commensals of differing growth stages on Stichopus. However, as has been said, although one may often find on a single Evasterias a number of young commensals, one rarely if ever finds more than one large Arctonoë on a starfish.

As yet no evidence (of brooding young, etc.) has appeared that would lead the writers to believe that the entire life-history of the worms is passed on the host. It is possible that the free-living larval stage may be of short duration. Ultimately, it must be determined at what stage and by what mechanism commensals find the host.

Summary

1. Water from an aquarium from which a host *Evasterias troschelii* has been removed 24 hours before no longer possesses an attraction for commensal *Arctonoë fragilis*. Similar results were obtained five hours after removal.

2. Water from an aquarium from which a host Evasterias has been removed with difficulty, so that tube feet were torn from it, has no attraction for commensals almost immediately after removal of the host. Such water appears to repel them.

3. Water from an aquarium from which a host Evasterias has been removed with comparative ease (involving less disturbance to the starfish) retains its attraction for commensals immediately after removal, but shortly thereafter the attraction appears to weaken.

4. If a host Evasterias is suspended in a test aquarium in a dialyzing bag for two hours, at the end of this time not enough attractant has passed through to attract commensals.

5. Experiments to determine whether attractant will diffuse through a dialyzing bag in effective concentrations overnight may be unsuccessful because the abnormal conditions inside the bag affect the host in such a way that the response of commensals is negated even after the host has been released from the bag into the test aquarium.

6. Washed eviscerated integument of *Stichopus californicus* does not appear to attract its commensal, *Arctonoë pulchra*.

7. Washed eviscerated integument of Evasterias does not appear to attract its commensal.

8. Washed viscera of Stichopus appears to repel its commensal.

9. Ground-up washed preparations of viscera of Evasterias appear to repel its commensal.

10. Immature Evasterias do not exert as strong an attraction on commensals as adults, even after compensation has been made for difference in weight.

11. No chemical attraction for its commensal, *Halosydna brevisetosa,* paralleling that observed in specific echinoderm-annelid partnerships can be demonstrated in the terebellid *Amphitrite robusta,* in spite of the fact that the scale worm is limited to this and one other terebellid host.

12. Commensal Halosydna are neutral to light or mildly negatively phototactic. A positive tactile response to the tentacles and body of the host was found in these worms.

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